

Application of fluorescence lifetime imaging microscopy to monitor glucose metabolism in pancreatic islets *in vivo*: supplement

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**APPLICATION OF FLUORESCENCE LIFETIME IMAGING MICROSCOPY TO
MONITOR GLUCOSE METABOLISM IN PANCREATIC ISLETS IN VIVO:
SUPPLEMENTAL DOCUMENT**

Visualization 1. Brightfield video of islets in side the eye.

Table S1. Antibodies used for immunofluorescence

Antibody	Supplier/ Cat#	Dilution
Rabbit anti-insulin	Cell Signaling; Cat# 3014	1:400
Mouse anti-glucagon	Sigma-Aldrich, Cat#G2654	1:1000
Donkey anti-rabbit-FITC	Jackson Lab, Cat# 711-096-152	1:200
Donkey anti-mouse-Cy3	Jackson Lab, Cat# 715-166-150	1:200

Table S2. FLIM fitting results

Species		1 component fitting	2 components fitting	3 components fitting
INS	χ^2	298.39±243.59	5.71±2.87	2.12±0.80
	τ	$\tau=1.19\pm0.14$	$\tau_1=0.46\pm0.04$ $\tau_2= 2.31\pm0.10$	$\tau_1=0.40$ $\tau_2=1.41\pm0.10$ $\tau_3=5.39\pm0.96$
Islets	χ^2	441.96±389.42	14.37±12.44	3.69±2.36
	τ	$\tau=1.39\pm0.14$	$\tau_1=0.49\pm0.03$ $\tau_2= 2.44\pm0.11$	$\tau_1=0.40$ $\tau_2=1.39\pm0.08$ $\tau_3=5.32\pm0.37$

Data is Mean±SEM.

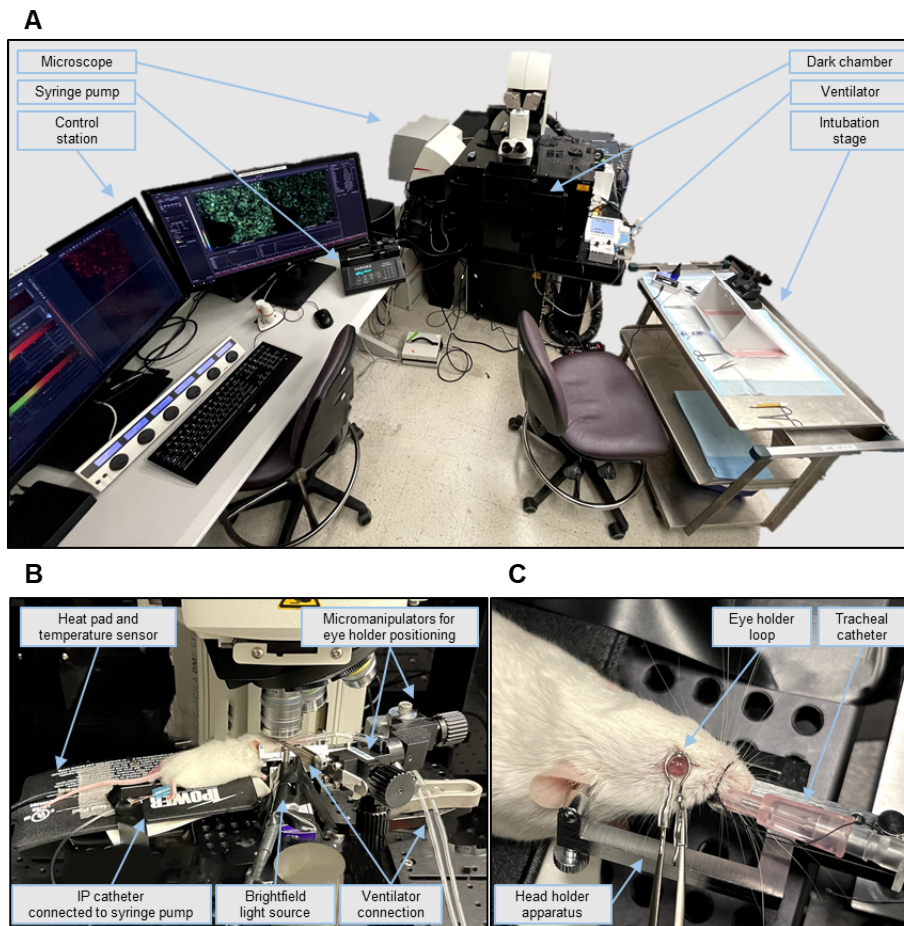


Fig. S1. **HARDWARE AND ANESTHESIA SETUP FOR IN VIVO FLIM IMAGING OF LIVE ISLETS GRAFTED TO THE IRIS IN MICE.** FLIM imaging of live islets grafted to the iris, enables monitoring glucose-stimulated islet metabolism in vivo. (A) To facilitate the in vivo FLIM studies we placed a bench top equipped for induction of anesthesia adjacent to the imaging station. (B) After anesthesia, the mouse is positioned head-up oriented to the microscope objectives. (C) The eye is stabilized by a metal loop gently applied with forceps to the corneoscleral junction. This diminishes movement artifacts associated with breathing.

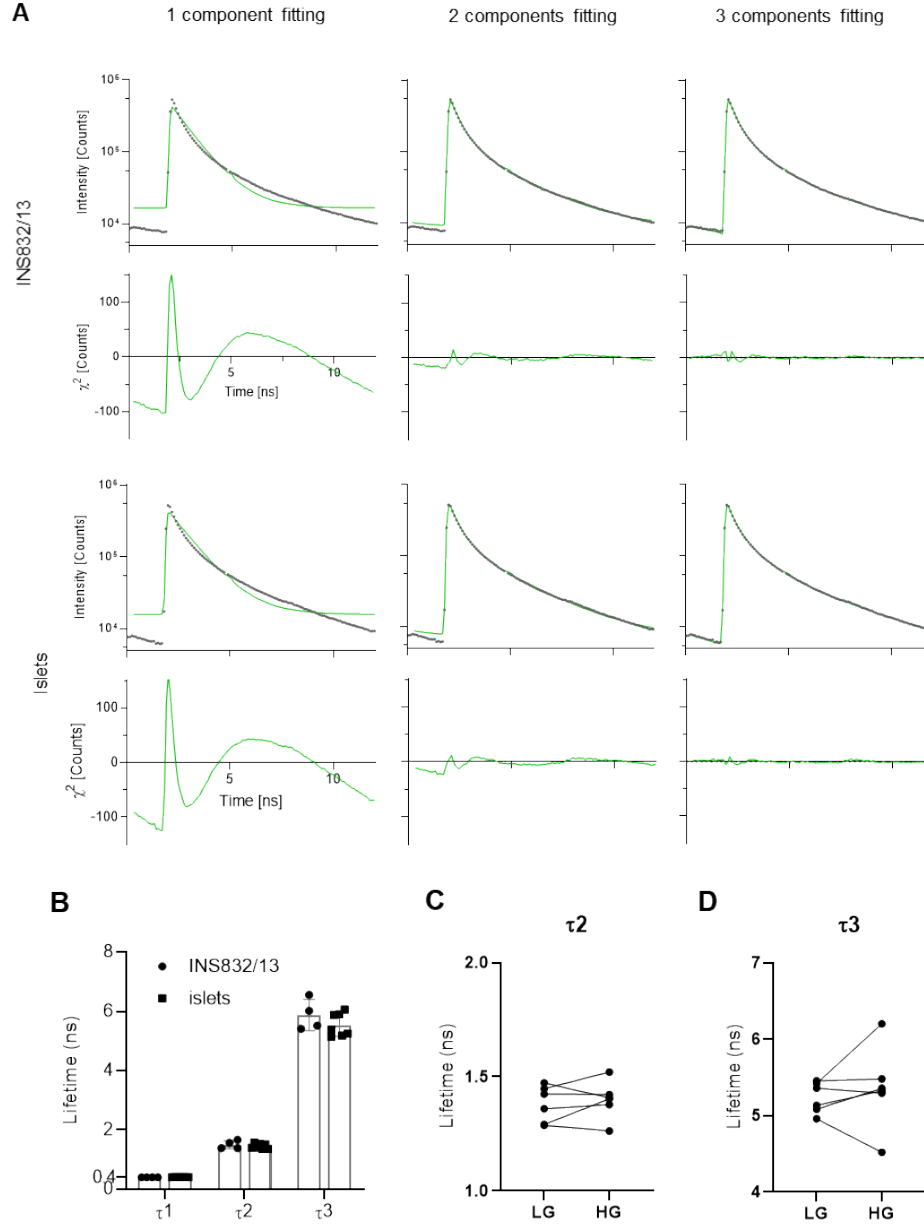


Fig. S1. 3-component fitting reveals enhanced OxPhos in engrafted islets under glucose stimulation. A. 3-component fitting resulted in smaller τ_2 than 1 component fitting in both INS832/13 cells and transplanted islets. Black dots, original data; green, fitted results. B. In 3 component fitting, both τ_2 and τ_3 showed no differences between INS832/13 cells and transplanted islets. Data is presented as Mean \pm SD. INS832/13, n=4; islets, n=6. C, D. Glucose stimulation did not affect τ_2 or τ_3 lifetime in islets, n=6.